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Impact of true fetal mosaicism on prenatal screening and diagnosis

Dear Editor,

Over the past decade, the non-invasive prenatal test (NIPT) has increasingly been used as a method for prenatal screening for trisomy 21 (T21) and other aneuploidies, complementing the traditional approach of first trimester screening (FTS). FTS comprises ultrasound of the nuchal thickness and blood test to measure the levels of maternal serum free-β-human chorionic gonadotropin and pregnancy-associated plasma protein-A (PAPP-A). FTS has been quoted to produce a sensitivity of 0.998 and 0.977 for T21 and T18, respectively, with a sensitivity of 0.900 for T13 in high-risk populations.[1] False positive rate was <1%[2] and false negative rate ranges between 0.02% and 0.26%.[3] Comparatively, the FTS test has a sensitivity of 90% at a false positive rate of 5%.[4]

We recently encountered a patient with a high-risk FTS and a low-risk NIPT who underwent chorionic villus sampling (CVS), which confirmed trisomy of the long arm and monosomy of the short arm of chromosome 18. This resulted in a mid-trimester termination of pregnancy.

This is a rare case of a false negative NIPT result. The basis of NIPT lies in the extraction of cell-free fetal DNA (cffDNA) circulating in the maternal serum, derived from placenta cytotrophoblast cells. In order to achieve adequate sequencing and analysis, the cell fetal fraction (FF), defined as the amount of cffDNA divided by the amount of total cell-free DNA, must be more than 4%. Factors affecting the FF include maternal weight and gestation age at time of test with a lower FF in higher maternal weight and lower gestational age.[2]

The theoretical explanation for false negative and false positive results lies in the fact that cffDNA is mainly derived from the apoptosis of the placenta cytotrophoblast and syncytiotrophoblast cells, which may be discordant with the true fetal karyotype due to mosaicism.[2,5] This cytotrophoblast layer is derived from the trophoblast of the blastocyst, whereas cells of the mesenchymal core and fetus are derived from the epiblast of the inner cell mass.

General mosaicism occurs when the aneuploidy occurs in the first days of embryonic development prior to any cellular differentiation (i.e. in a preimplantation embryo). Early embryos have a mosaicism rate of 65–70%.[6] However, not all abnormal cell lines continue to propagate during development because euploid cells proliferate more quickly than aneuploidy cells. Confined placental mosaicism (CPM) is a phenomenon when the chromosomally abnormal cell line is confined within the cytotrophoblast and the mesenchymal core of the chorionic villus, while the fetus itself has a normal karyotype.[3]

False positive NIPT results may arise due to CPM Type 1 or Type 3,[3,5] where the fetus has a normal karyotype but the cytotrophoblast cells are abnormal (Table 1). The chance of obtaining a false positive NIPT result due to CPM increases with higher percentage of mosaicism, greater fetal fraction and method of performing NIPT (counting or single-nucleotide polymorphism-based methods).[7] False negative NIPT results due to mosaicism are largely due to true fetal mosaicism (TFM) type 5,[5] where the cytotrophoblast layer has a normal karyotype while the mesenchymal core and fetus have an abnormal karyotype (Table 1).

The consensus statement released by the International Society of Ultrasound in Obstetrics and Gynecology (ISUOG) in 2014 recommended that all women should be offered a first trimester ultrasound scan, regardless of their intention to undergo NIPT.[8] The use of NIPT can either be performed as a first line screening test or as an alternative to invasive testing following an abnormal or "intermediate risk" result on combined screening test. However, it recommended the cautious use of NIPT as most guidelines endorse NIPT only for high-risk populations, and usage of NIPT in lower-risk populations may result in lower positive predictive values.[8] The ISUOG also recommended that the use of NIPT should not replace invasive diagnostic tests in patients with trisomy risk of more than 1 in 10 on FTS as only 70% of chromosomal abnormalities in this group of patients are trisomy 21, 18 or 13. In our case, if we had taken NIPT alone without FTS, we may not have picked up the abnormality until the fetal anomaly scan usually performed around 20 weeks of gestation.

All abnormal results from NIPT should hence be confirmed with diagnostic testing. This can either be in the form of a chorionic villus sampling or an amniocentesis, depending on the involved chromosomal aberration. Chorionic villus sampling involves analysis

Table 1. Summary of the effects of various types of CPM and TFM on NIPT results[a]

Types of mosaicism		Cytotrophoblast (direct preparation or short-term culture)	Mesenchyme (long-term culture)	Amniocytes	Expected NIPT result
CPM	I	Abnormal	Normal	Normal	False positive
	II	Normal	Abnormal	Normal	True negative
	III	Abnormal	Abnormal	Normal	False positive
TFM	IV	Abnormal	Normal	Abnormal	True positive
	V	Normal	Abnormal	Abnormal	False negative
	VI	Abnormal	Abnormal	Abnormal	True positive

CPM: confined placental mosaicism; NIPT: non-invasive prenatal test; TFM: true fetal mosaicism
[a] Grati FR. Chromosomal Mosaicism in Human Feto-Placental Development: Implications for Prenatal Diagnosis. J Clin Med 2014;3:809-37.

of both the cytotrophoblast and mesenchymal core, while amniocentesis involves sampling amniocytes. If the NIPT is high risk for trisomy 13, 18 or 21, then CVS is a reasonable option as the result is representative of the fetal karyotype in around 97% of cases.[9] Patients should however be counselled of the 3% risk of receiving a mosaic result that would need further confirmatory testing with amniocentesis. If NIPT is high risk for sex chromosome aneuploidy, then amniocentesis is recommended over CVS as the chance of CPM is higher.[9]

Our case illustrates the importance of pre-test counselling emphasising the limitations of prenatal screening test and alternative options. Patients should be aware of the possibility of a false negative screening test and the implications on the management of the pregnancy. Fetal anomaly screening scans and regular antenatal follow-up scans remain as a second line of assessment for these patients in detecting fetal anomalies that may trigger further invasive diagnostic testing. A delayed detection of fetal chromosomal abnormalities poses significant distress to parents deciding on late termination of pregnancy, and care issues for parents where abnormalities are only detected at birth. Parental karyotyping for assessment of recurrence risks should also be offered for post-pregnancy counselling. Lastly, clinicians should understand the basis of NIPT and its limitations such as in this scenario of TFM so as to allow appropriate counselling.

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